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Part V

Environmental Protection Agency

40 CFR Part 141

National Primary Drinking Water Regulations; Analytical Techniques; Coliform Bacteria; Final Rule

ENVIRONMENTAL PROTECTION AGENCY

40 CFR Part 141

[WH-FRL-4012-9]

National Primary Drinking Water Regulations; Analytical Techniques; Coliform Bacteria

AGENCY: Environmental Protection Agency (EPA).

ACTION: Final rule.

SUMMARY: In an earlier notice (54 FR 27544; June 29, 1989), EPA approved the use of the Minimal Medium ONPG–MUG (MMO–MUG) Test for total coliform analysis for compliance with the maximum contaminant level (MCL) for total coliforms under the Safe Drinking Water Act (SMWA). (ONPG is ortho-nitrophenyl-β-D-galactopyranoside; MUG is 4-methylumbelliferyl-β-D-glucuronide.) On January 8, 1991, EPA deferred approval of the MMO–MUG Test for *Escherichia coli (E. coli)* detection. The Agency also restricted transferring cultures (i.e., subculturing) from total coliform-positive MMO–MUG tests. Today's action relieves this restriction.

EFFECTIVE DATE: January 15, 1992.

ADDRESSES: The supporting documents cited in the reference section of this notice and other pertinent information are available for review at EPA's Drinking Water docket, 401 M Street, SW., Washington, DC 20460. For access to the docket materials, call (202) 260–3027 on Monday through Friday, excluding Federal holidays, between 9 a.m. and 3:30 p.m. Eastern Time for an appointment.

FOR FURTHER INFORMATION CONTACT: Paul S. Berger, Ph.D., Office of Ground Water and Drinking Water (WH–550D), Environmental Protection Agency, 401 M Street, SW., Washington, DC 20460, telephone (202) 260–3039; or the Safe Drinking Water Hotline, telephone (800) 426–4791. The Safe Drinking Water Hotline is open Monday through Friday, excluding Federal holidays, from 8:30 a.m. to 5 p.m. Eastern Time.

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I. Statutory Authority

The SDWA requires EPA to promulgate National Primary Drinking Water Regulations (NPDWRs) which include MCLs or treatment techniques (section 1412). NPDWRs also contain "criteria and procedures to assure a supply of drinking water which dependably complies with such maximum contaminant levels; including quality control and testing procedures to insure compliance with such levels * * *" (section 1401(1)(D)). In addition, section 1445(a) of the SDWA authorizes the Administrator to require monitoring to assist in determining whether persons are in compliance with the requirements of the SDWA. EPA's promulgation of analytical techniques is authorized under these sections of the SDWA. EPA has promulgated analytical techniques for all currently regulated drinking water contaminants; persons must use one of the approved analytical techniques for determining compliance with the MCLs (see 40 CFR 141.21–30). Today's action removes a constraint in the use of a previously approved method for detecting total coliform bacteria.

II. Regulatory Background

On June 19, 1989, EPA promulgated revised regulations for total coliforms (54 FR 27544, June 29, 1989), with an effective date of December 31, 1990. Paragraph 141.21(f) of those regulations approves four analytical methods for testing for the presence of total coliforms, including the MMO–MUG Test. Paragraph 141.21(e) requires public water systems to test all total coliform-positive cultures for the presence of either fecal coliforms or *E. coli*. EPA proposed three analytical methods for *E. coli* on June 1, 1990, and promulgated two of them on January 8, 1991.

The Agency deferred final action on one of the proposed methods for *E. coli* detection, the MMO–MUG Test (also referred to as the Autoanalysis Colilert System), because public commenters raised questions about its ability to detect low densities of environmentally stressed *E. coli*. This issue, the false-negative rate, is not addressed by today's action. Data provided by one commenter led the Agency to consider whether to allow transfer (subculture) from a total coliform-positive (but *E. coli*-negative) MMO–MUG tube to an EPA-approved *E. coli* medium. In the preamble, the Agency indicated that it would consider additional data to determine if such transfers should be permitted. The Agency's decision to give further consideration to subculturing effectively meant that laboratories could not use the MMO–MUG Test for determining compliance with the total coliform rule. This subculture issue is the subject of today's action.

III. Discussion

Today's action removes the limitation imposed by the Agency's further study on subculturing, and allows a laboratory to transfer a total coliform-positive, MUG-negative (i.e., *E. coli*-negative) culture by the MMO–MUG Test to an EPA-approved *E. coli* test. This is accomplished by minor adjustments to the already approved subculturing procedures specified in the January 8 rule for other tests.

The MMO–MUG Test is designed to identify both total coliforms and *E. coli* in a single container. The *E. coli* portion of the test is based on the ability of *E. coli* to produce the enzyme beta-glucuronidase, which hydrolyzes the 4-methylumbelliferyl-beta-D-glucuronide (MUG) contained in the medium to form 4-methylumbelliferone, which fluoresces when exposed to ultraviolet light (366 nm). The MUG reaction is also the basis for the EC+MUG test and Nutrient Agar+MUG test, both of which were approved by EPA for *E. coli* testing in the January 8, 1991, rule.

As stated above, EPA deferred the use of subculturing from the MMO–MUG Test until data were available to show that an initially low density of stressed *E. coli* would grow in the MMO–MUG Test medium, within the prescribed 28 hour incubation time, to a sufficiently high density. A high density is needed to allow a laboratory to transfer (i.e., subculture) and *E. coli*-positive, but MUG-negative, culture to an approved *E. coli* test, using a pipet, loop, or other standard microbiological transfer technique.

EPA developed a testing protocol to examine the transfer question, and this protocol was reviewed by EPA's Science Advisory Board. The protocol describes an approach for determining if initially low numbers of chlorine-injured *E. coli* in total coliform-positive cultures by the MMO–MUG Test could be transferred to and detected by the EPA-approved EC Medium+MUG. EPA funded a study using the transfer protocol which indicated that initially low levels of *E. coli* do reach sufficiently high levels after 28 hours of incubation to allow for successful transfer. This is true for both MUG-positive and MUG-negative MMO–MUG tests. The protocol used and the final report of the study are in

the EPA's Drinking Water Docket for *E. coli*.

In this study, Dr. Pipes of Drexel University found that all MUG-positive cultures from the MMO–MUG Test (total of 88) were also MUG-positive in EC Medium + MUG. These data support the view that few false-positives are associated with the MMO–MUG Test. Of the 32 MUG-negative cultures from the MMO–MUG Test, 10 and 11 cultures were MUG-positive in EC Medium + MUG using a calibrated loop (0.01 ml) or pipet (0.1 ml), respectively, for transfer. Based on Dr. Pipes' statistical analysis of the data, it is likely that most or all of the remaining MUG-negative MMO–MUG cultures (total of 21) were *E. coli*-negative. These data indicate that MUG-negative, but *E. coli*-positive, cultures from the MMO–MUG medium can be successfully transferred.

Based on this analysis, EPA concludes that stressed *E. coli* in MUG-negative cultures can grow to a density sufficient for successful transfer by a pipet. Of the eleven MUG-negative MMO–MUG cultures that were MUG-positive in EC Medium + MUG, however, only five were MUG-positive in EC Medium + MUG medium when the original MUG-negative MMO–MUG cultures were diluted by a factor of 10 or 100 before transfer. This suggests that, in some cases, the density of initially stressed *E. coli* only barely reached levels that allowed for successful transfer.

Given the data above, EPA believes that *E. coli* usually will either produce a MUG-positive reaction after 28 hours of incubation, or, if MUG-negative, can be successfully subcultured to EC Medium + MUG. For this reason, today's action allows laboratories to transfer a total coliform-positive MMO–MUG Test culture to an EPA-approved *E. coli* medium. The Agency cautions, however, that any such transfer is to be conducted on a 28-hour culture, rather than a 24-hour culture. EPA believes a 28-hour incubation time is sufficient to allow initially low densities of stressed *E. coli* to reach sufficiently high densities to allow transfer by standard microbiological procedures.

As stated above, EPA's primary uncertainty about the MMO–MUG Test is the false-negative rate. As discussed in the preamble to the notices of June 1, 1990, and January 8, 1991, and in the Comment/Response document to the final rule, EPA believes the false-positive rate for the *E. coli* portion of the MMO–MUG Test is low. For this reason, EPA believes that a total coliform-positive culture which fluoresces (i.e., MUG-positive) must be considered *E. coli*-positive. Thus, the Agency does not believe it necessary for a laboratory to subculture a MUG-positive result to an already approved *E. coli* medium.

IV. Notice and Comment

As noted above, comments on the *E. coli* methods focused on the false-negative rate of the MMO–MUG test in certain circumstances. As a result of these concerns, EPA deferred a decision on the use of the MMO–MUG test to detect *E. coli* pending additional analysis of this false-negative issue. Recognizing the continuing interest by the public in this issue, the Agency indicated in the January 8, 1991, notice, that it would present any new data in a notice of availability and seek public comment on whether to approve the MMO–MUG test as proposed earlier. One commenter questioned whether transfer from an MMO–MUG test to an EPA-approved medium should be allowed. While the January notice indicated that additional data on this subculturing issue would be provided for comment, the Agency has since concluded that for the reasons discussed below, public comment is unnecessary.

The comment on subculturing raised a minor technical issue. The Agency developed additional data to address the concerns raised by the commenter. Based on analysis of those data, the Agency has concluded that minor adjustments to the existing transfer methodology permit transfer from the MMO–MUG Test to an EPA-approved medium for *E. coli* detection. These adjustments do not substantially alter the framework of the already-approved transfer methodology. Moreover, these adjustments remove the barrier preventing use of the MMO–MUG Test for total coliform detection, a method already approved by the Agency.

V. Effective Date

This rule is effective on the date of publication, rather than 30 days from publication, as provided by the Administrative Procedure Act. EPA believes there is good cause for this rule to be effective immediately. This amendment relieves a restriction in the revised total coliform rule, and will benefit public water systems by allowing them to use an analytical method for total coliforms that the Agency has already approved. This approved method has the advantage of ease of application and therefore will provide an overall public benefit.

VI. Regulation Assessment Requirements

A. Executive Order 12291

Executive Order 12291 requires EPA to judge whether a regulation is "major" and, if so, to prepare a regulatory impact analysis. A rule is considered major if it has an economic impact of $100 million or more, causes a significant increase in cost or prices, or any of the other adverse effects described in the Executive Order. Since the objective of this rule is merely to allow a previously approved analytical method for total coliforms to be used, EPA has determined that this action is not a major rule within the meaning of the Executive Order. Water systems/laboratories may use the MMO–MUG Test for total coliforms or continue using other previously-approved methods. Therefore, there will not be any adverse economic impacts.

This notice was submitted to the Office of Management and Budget for its review under the Executive Order.

B. Regulatory Flexibility Analysis

The Regulatory Flexibility Act requires EPA to explicitly consider the effect of proposed regulations on small entities. If there is a significant effect on a substantial number of small systems, means should be sought to minimize the effects. The Small Business Administration defines a small water utility as one which serves fewer than 3,300 people. Under this definition, this rule would affect about 200,000 small systems.

This final rule is consistent with the objectives of the Regulatory Flexibility Act because it will not have a significant economic impact on small entities. The rule allows the use of an already approved analytical method for total coliform analysis. Since use of the *E. coli* tests is optional, and EPA is not promulgating any new requirement, the Agency believes that the impact of this notice would not have a significant effect on a substantial number of small entities.

C. Paperwork Reduction Act

This rule contains no information collection requirements and consequently is not covered by the Paperwork Reduction Act, 44 U.S.C. 3501 et seq.

D. Science Advisory Board, National Drinking Water Advisory Council, and Secretary of Health and Human Services

In accordance with section 1412 (d) and (e) of the Safe Drinking Water Act,

the Agency consulted with the Science Advisory Board, National Drinking Water Advisory Council, and the Secretary of Health and Human Services and took their comments into account in developing the proposed rule on *E. coli* methods. In addition, the Agency submitted a copy of the transfer protocol to the Science Advisory Board for review, and revised it according to their comments.

List of Subjects in 40 CFR Part 141

Chemicals, Microorganisms, Indians—land, Intergovernmental relations, Radiation protection, Reporting and recordkeeping requirements, Water supply.

VII. References

Pipes, W. 1991. The transferability of *Escherichia coli* from MMO–MUG media for detection in drinking water samples. Report submitted to the U.S. Environmental Protection Agency, Office of Ground Water and Drinking Water.

Dated: January 7, 1992.

William K. Reilly,

Administrator.

For the reasons set out in the preamble, part 141 of title 40 of the Code of Federal Regulations is amended as follows:

PART 141—NATIONAL PRIMARY DRINKING WATER REGULATIONS

1. The authority citation for part 141 continues to read as follows:

Authority: 42 U.S.C. 300f, 300g–1, 300g–2, 300g–3, 300g–4, 300g–5, 300g–6, 300j–4 and 300j–9.

2. Section 141.21 is amended by redesignating paragraph (f)(7) as paragraph (f)(8), and by adding a new paragraph (f)(7) to read as follows:

§ 141.21 Coliform sampling.

* * * * *

(f) * * *

(7) If a system uses the MMO–MUG Test for total coliform detection, it must test all total coliform-positive cultures for fluorescence. To test for fluorescence, use an ultraviolet light (366 nm) in the dark after incubating the tube or container at 35 ± 0.5°C for 24–28 hours. If fluorescence is observed, the sample is *E. coli*-positive. If fluorescence is not observed, transfer a 0.1 ml, 28-hour culture to EC Medium + MUG with a pipet. The formulation and incubation conditions of EC Medium + MUG, and observation of the results are described in paragraph (f)(6)(i) of this section.

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